Secretome of in vitro cultured human embryos contains extracellular vesicles that are uptaken by the maternal side

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Abstract: Communication between embryo and maternal endometrium occurs during a specific time frame in which implantation is possible. Here we demonstrate for the first time that conditioned media from non-manipulated human embryos cultured in vitro for 3 days or up to the blastocyst stage contain extracellular vesicles (EVs) with a diameter of 50 to 200 nm and bearing the traditional microvesicle and exosome marker proteins CD63, CD9 and ALIX. The embryonic origin of these EVs has been confirmed by the presence of stemness gene transcripts and their enrichment in the non-classical HLA-G protein. NANOG and POU5F1 transcripts were shown to be contained in vesicles deriving from embryos at different stages of development. In line with a higher detection rate of the HLA-G protein in blastocysts compared to cleavage stage embryos, a significantly higher amount of HLA-G was found in vesicles accumulated in spent media from day 3 to day 5 of development compared to those isolated from the earlier stage. Uptake of dye-labeled embryo-derived EVs by human primary endometrial epithelial and stromal cells was also demonstrated with a fluorescence intensity signal significantly higher for cells treated with vesicles derived from blastocysts. Based on these findings, EV exchange may be suggested as an emerging way of communication at the maternal-fetal interface.

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Extraintestinal Manifestations of Pediatric Inflammatory Bowel Disease: Prevalence, Presentation, and Anti-TNF Treatment

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ABSTRACT

Background: There is a paucity of data on extraintestinal manifestations (EIM) and their treatment in pediatric patients with inflammatory bowel disease (IBD).

Methods: Since 2008, the Pediatric Swiss IBD Cohort Study has collected data on the pediatric IBD population in Switzerland. Data on 329 patients were analyzed retrospectively.

Results: A total of 55 patients (16.7%) experienced 1–4 EIM (39 Crohn disease, 12 ulcerative colitis, and 4 IBD-unclassified patients). At IBD onset, presence of EIM was more frequent than in the adult population (8.5% vs 5.0%, $P = 0.014$). EIM were more frequent in Crohn disease when compared to ulcerative colitis/IBD-unclassified (22.5% vs 10.3%, $P = 0.003$). The most prevalent EIM were peripheral arthritis (26/329, 7.9%) and aphthous stomatitis (24/329, 7.3%). Approximately 27.6% of all EIM appeared before IBD diagnosis. Median time between IBD diagnosis and occurrence of first EIM was 1 month (−37.5–149.0). Thirty-one of the 55 patients (56.4%) were treated with 1 or more anti–tumor necrosis factor (TNF) agents. IBD patients with EIM were more likely to be treated with anti-TNF compared to those without (56.4% vs 35.0%, $P = 0.003$). Response rates to anti-TNF depended on underlying EIM and were best for peripheral arthritis (61.5%) and uveitis (66.7%).

Conclusions: In a cohort of pediatric patients with IBD, EIM were frequently encountered. In up to 30%, EIM appeared before IBD diagnosis. Knowledge of these findings may translate into an increased awareness of underlying IBD, thereby decreasing diagnostic delay. Anti-TNF for the treatment of certain EIM is effective, although a substantial proportion of new EIM may present despite ongoing anti-TNF therapy.

Key Words: anti–tumor necrosis factor, arthritis, extraintestinal manifestations, inflammatory bowel disease, uveitis

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What Is Known

- Extraintestinal manifestations are frequently observed in adult patients with inflammatory bowel disease.
- Little is known about extraintestinal manifestation in the pediatric inflammatory bowel disease population.

What Is New

- Extraintestinal manifestations are common in pediatric patients with inflammatory bowel disease.
- Epidemiology and presentation of extraintestinal manifestation seem to be similar in the pediatric and adult inflammatory bowel disease population.
- In up to 30% of those patients presenting with extraintestinal manifestation, extraintestinal manifestation appear before inflammatory bowel disease diagnosis.
- Type of extraintestinal manifestation may affect the responses to anti–tumor necrosis factor. Best rates were seen for peripheral arthritis and uveitis.

Inflammatory bowel diseases (IBDs) are chronic inflammatory disorders of the gastrointestinal tract and can be classified into the 2 main subtypes Crohn disease (CD) and ulcerative colitis (UC)

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(1). As IBDs are systemic diseases, which can involve multiple organ systems, extraintestinal manifestations (EIM) are frequently observed affecting up to 50% of the adult IBD population (2–6). In a non-negligible proportion, EIM appear even before IBD diagnosis is established (7). Although some reviews broaden the concept of EIM to non–IBD-specific autoimmune disorders such as thyroid disease or vitiligo and IBD-related complications such as osteopathy or nephrolithiasis, the typical EIM involve the following 4 organs: skin (erythema nodosum [EN], pyoderma gangrenosum [PG], psoriasis, aphthous stomatitis), joints (peripheral arthritis, axial arthropathy), biliary tract (primary sclerosing cholangitis [PSC]), and eyes (uveitis) (7). Most of EIM parallel intestinal disease activity (8–12), are more common in CD than in UC, and are more frequently observed with longer IBD duration (13). In addition, up to one quarter presents with more than 1 EIM (8). Morbidity and mortality are considerably affected (14,15). Although pathogenesis remains mostly elusive, intestinal and extraintestinal IBDs seem to share tumor necrosis factor (TNF)-dependent mechanisms (16) and several studies and case reports were able to demonstrate beneficial effect of anti-TNF treatments (17–23). EIM (such as arthritis and paradoxical psoriasiform reactions) can, however, also resemble adverse effects of anti-TNF therapy (24).

Although knowledge of prevalence, appearance, and possible treatment options for EIM in adults is increasing, it is still limited in the pediatric IBD population. Recent studies have shown higher rates of EIM at IBD onset compared with adults with peripheral arthritis and aphthous stomatitis being the most prevalent (25,26). To our knowledge, so far no study has addressed the chronological order of EIM appearance in respect to IBD diagnosis or the influence of anti-TNF treatment on the evolution and development of EIM in a pediatric IBD cohort. Given this paucity of data, we aimed to assess the frequency and type of EIM, chronological order of appearance of EIM, and the use of and response to anti-TNF treatment in the Pediatric Swiss IBD Cohort Study (PSIBDCS).

METHODS

Patients

The PSIBDCS is a nationwide substudy of the Swiss IBD Cohort Study (SIBDCS) including all regions of Switzerland (27). Enrollment started in 2008. The SIBDCS and its substudy are supported by the Swiss National Science Foundation and are approved by the local ethics committees of the participating centers. Patients are included in the PSIBDCS if they are 18 years or younger. Additional inclusion criteria and regular assessment scheme (baseline and annual follow-up questionnaires) have been discussed elsewhere (28). Patients were recruited at University Hospitals, community hospitals, and large private practices throughout Switzerland. A total of 329 pediatric patients are currently included. All 329 patients were retrospectively analyzed for the purpose of the present study.

Definition of Extraintestinal Manifestation and Anti–tumor Necrosis Factor Outcome

All EIM had to be diagnosed by clinical experts: diagnosis of skin manifestations was established by a dermatologist, joint affections by a rheumatologist, eye manifestations by an ophthalmologist, and PSC by a gastroenterologist. We analyzed the following EIM: peripheral arthritis, uveitis, PG, EN, aphthous stomatitis, axial arthropathy, psoriasis, and PSC. Diagnosis of EIM relied on previously published criteria (7,25,29). We did not consider anemia, glaucoma, and pancreatic involvement as EIM because it may also be considered as complication of IBD therapy (25). Evolution of EIM under anti-TNF treatment was judged according to the physician's global assessment, which was based on patient history and clinical findings. This anti-TNF response was classified into the following 3 categories: clinical improvement, stable disease course unaffected by anti-TNF treatment, and clinical worsening.

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Data Collection and Management

Completed patient and physician questionnaires (baseline and annual follow-up) were sent to the datacenter of the SIBDCS located at the Institute of Social and Preventive Medicine, University of Lausanne, Switzerland, where data were validated by the responsible data manager and finally entered into a Microsoft Access database (Access 2000; Microsoft Switzerland Ltd. Liab. Co., Wallisellen, Switzerland). Baseline questionnaire at enrollment and annual follow-up questionnaires included demographic data, IBD subtype and diagnosis, disease localization according to international guidelines, prior and current medications, and past and present EIM. Electronic and written charts of those patients, who reported past or present EIM, were additionally reviewed by clinical experts to extract exact appearance of EIM in relation to IBD diagnosis, EIM subtype, chronological order of appearance of multiple EIM, and evolution of EIM in response to anti-TNF treatment. All 329 patients in the PSIBDCS were eligible. For detailed analysis, only those patients with 1 or more EIM were included. For a comparison between the pediatric and adult population, data from the SIBDCS were used according to a recent study conducted by Vavricka et al (7).

Statistical Analysis

For all statistical analyses, IBM software SPSS version 23.0.0 (2014 SPSS Science, Inc., Armonk, NY) was used. Metric data are shown as medians with their total range. Categorical data are depicted as percentage of the group total. Comparison between categorical variables was performed by using Chi-square test or Fisher exact test, if sample size was low ($n < 10$). A 2-sided P value of <0.05 was considered statistically significant. The association between potential predicting factors and positive anti-TNF outcome was analyzed by means of logistic regression.

RESULTS

Patient Demographics

Of the 329 pediatric patients with IBD included in the study, 173 (52.6%) had CD and 156 (47.4%) had UC/IBD-unclassified (IBD-U), 148 patients were girls (45.0%). Median age at enrollment was 14 years (0–17) and median age at IBD diagnosis was 12 years (0–16). The patients had been experiencing IBD for a median of 3 years (0–16). Patient demographics according to IBD subtype (including disease localization and received medications) are summarized in Table 1. Of the 329 patients, 55 presented with 1 or more EIM (16.7%). The characteristics of those patients are depicted in Table 2.

Frequency and Types of Extraintestinal Manifestation

Of the 55 patients with at least 1 EIM, 39 patients had CD (39/55, 70.9%), 12 patients had UC (12/55, 21.8%), and 4 patients had IBD-U (4/55, 7.3%) as their underlying condition. Of these 55 patients with EIM, 39 (70.9%), 12 (21.8%), 3 (5.5%), and 1 patient (1.8%) reported 1, 2, 3, and 4 EIM, respectively. At IBD onset, presence of EIM was more frequent than that in the adult population (28/329, 8.5% vs 62/1249, 5.0%, $P = 0.014$). EIM were more frequently observed in patients with CD (39/173, 22.5%) when compared to patients with UC/IBD-U (16/156, 10.3%, $P = 0.003$). The most prevalent EIM were peripheral arthritis (26/329, 7.9%) and aphthous stomatitis (24/329, 7.3%), followed by uveitis (6/329, 1.8%), EN (5/329, 1.5%), axial arthropathy (5/329, 1.5%), psoriasis (4/329, 1.2%), PSC (4/329, 1.2%), and PG (2/329, 0.6%). Peripheral arthritis, axial arthropathy, and EN were less frequently encountered among pediatric patients with IBD compared to adult patients (7.9% vs 20.5%, $P < 0.001$; 1.5% vs 4.8%, $P = 0.008$; and 1.5% vs 3.7%, $P = 0.048$). Frequency of other EIM was comparable between the 2 populations. For a detailed overview see Figure 1. Although 27.6% of all EIM (21/76) appeared before the diagnosis of IBD, the majority of EIM appeared once IBD diagnosis was established (42/76, 55.3%). The remaining proportion of EIM (12/76, 15.8%) was first observed at the time of establishment of IBD diagnosis. Data from 1 EIM were missing. So, at the time of IBD diagnosis, 28 of the 55 patients presented with a total of 33 EIM (observed before or at the time of IBD diagnosis); aphthous stomatitis and peripheral arthritis were again the most frequently observed EIM (15/28, 53.6% and 10/28, 35.7%, respectively). For a synopsis over the phenotypic features at IBD diagnosis see Supplemental Digital Content 1, Table 1 (*http://links.lww.com/MPG/A827*). Details about frequency and type of EIM according to IBD subtype are shown in Table 2.

Distribution of Different Extraintestinal Manifestation According to Their Chronological Appearance

Aphthous stomatitis was the most prevalent EIM (21/55, 38.2%), which appeared as first EIM ($n = 55$), followed by peripheral arthritis (19/55, 34.5%) and uveitis (4/55, 7.3%). If the patient was diagnosed with a second EIM ($n = 16$), peripheral arthritis (5/16, 31.3%), EN (3/16, 18.8%), aphthous stomatitis (2/16, 12.5%), and axial arthropathy (2/16, 12.5%) were most frequent. In those patients presenting with a third EIM ($n = 4$), occurrence of the following EIM were reported: peripheral arthritis (1/4, 25.0%), uveitis (1/4, 25.0%), aphthous stomatitis (1/4, 25.0%), and psoriasis (1/4, 25.0%). Median time between IBD diagnosis and occurrence of first EIM was 1 month (range −37.5–149.0). Figure 2 illustrates the chronological order of appearance of each individual EIM in relation to the time of IBD diagnosis (in months). Peripheral arthritis appeared before IBD diagnosis in 28.0% (7/25, exact appearance in 1 case unknown), uveitis in 16.7% (1/6), EN in 20% (1/5), axial arthropathy in 40.0% (2/5), and aphthous stomatitis in 29.2% (7/24). All cases of psoriasis (4/4, 100%) and PG (2/2, 100%) appeared after IBD diagnosis were established. In addition, peripheral arthritis (64.0% vs 28.0%, $P = 0.011$) and uveitis (83.3% vs 16.7%, $P = 0.021$) were significantly more likely to appear after diagnosis of IBD than before establishment of IBD diagnosis. Median lag of time of appearance before IBD diagnosis in the group of patients in whom EIM preceded IBD diagnosis was −5.0 months (range −37.5 to −0.4).

Type of Anti–tumor Necrosis Factor Treatment and Treated Extraintestinal Manifestation

Anti-TNF therapy was initiated in 31 of the 55 patients with EIM (56.4%). Most of them were treated with a single anti-TNF agent (23/31, 74.2%), whereas 5 patients were treated with 2 and 3 patients with 3 different anti-TNF agents (5/31, 16.1% and 3/31, 9.7%, respectively). So, a total of 42 treatment courses were initiated. In 78.6% (33/42), anti-TNF treatment was started for the purpose of treating underlying IBD activity. In 3 cases (3/42, 7.1%), anti-TNF was solely initiated for the purpose of treating EIM; infliximab was started for peripheral arthritis ($n = 1$) and axial arthropathy ($n = 1$); and adalimumab was initiated for peripheral arthritis ($n = 1$). In 5 cases (5/42, 11.9%), anti-TNF was started for the purpose of treating both intestinal disease activity and EIM. In 1 case, exact indication for

TABLE 1. Patient demographics of the pediatric cohort

	CD patients	UC/IBD-U patients	All IBD patients
Number of patients	173 (52.6)	156 (47.4)	329 (100.0)
Sex			
Male	104 (60.1)	77 (49.4)	181 (55.0)
Female	69 (39.9)	79 (50.6)	148 (45.0)
Age at diagnosis in years	12, 10–14	11, 7–14	12, 9–14
(median, IQR, range)	0–16	0–16	0–16
Age at enrollment, y	14, 12–15	13, 11–15	14, 11–15
(median, IQR, range)	0–17	0–17	0–17
Age at latest follow-up, y	16, 14–17	16, 13–17	16, 13–17
(median, IQR, range)	0–18	0–18	0–18
Disease duration, y	3, 2–5	3, 1–6	3, 2–5
(median, IQR, range)	0–15	0–16	0–16
Diagnostic delay, mo	4.1, 2.0–8.1	3.0, 1.0–6.1	3.1, 2.0–7.1
(median, IQR, range)	0–83.2	0–59.9	0–83.2
Initial disease location (CD)			
L1	23 (13.3)	–	
L2	22 (12.7)	–	
L3	119 (68.8)	–	
L4 only	3 (1.7)	–	
Unknown/unclear	6 (3.5)	–	
Initial disease location (UC)			
E1	–	13 (8.3)	
E2	–	28 (18.0)	
E3/E4	–	104 (66.7)	
Unknown/unclear	–	11 (7.0)	
Fistulas			
Perianal fistula	24 (13.9)	–	
Other fistula	12 (6.9)	–	
Stenosis			
Any stenosis	22 (12.7)	–	
Medication ever received			
5-ASA	84 (48.6)	149 (95.5)	233 (70.8)
Antibiotics	64 (37.0)	46 (29.5)	110 (33.4)
Steroids	140 (80.9)	117 (75.0)	257 (78.1)
Immunomodulators	155 (89.6)	101 (64.7)	256 (77.8)
Anti-TNF	87 (50.3)	40 (25.6)	127 (38.6)

CD = Crohn disease; IBD-U = inflammatory bowel disease-unclassified; TNF = tumor necrosis factor; UC = ulcerative colitis.

anti-TNF treatment was not known. IBD patients presenting with EIM were more likely to be treated with anti-TNF compared to those without EIM (31/55, 56.4% vs 96/274, 35.0%, $P = 0.003$). The most frequently treated EIM were peripheral arthritis (16/42, 38.1%) and aphthous stomatitis (13/42, 31.0%), followed by axial arthropathy, uveitis, and EN. For details see Table 3. Under anti-TNF treatment, 23 EIM appeared in 19 of the 31 treated patients (19/31, 61.3%). Among those 23 EIM, peripheral arthritis (6 cases) and aphthous stomatitis (5 cases) were the most frequently reported. Three cases of psoriasis occurred under anti-TNF therapy, which can be considered as anti-TNF-induced psoriasiform skin lesions. Further reported EIM, which appeared under anti-TNF, were: uveitis (2 cases), PG (1 case), EN (2 cases), and axial arthropathy (1 case). In remaining 3 cases, the exact EIM subtype was unknown.

Clinical Evolution of Extraintestinal Manifestation Under Anti–tumor Necrosis Factor Treatment

Data on clinical outcome of anti-TNF treatment were available for 37 of the 53 treated EIM (69.8%). In the majority, EIM showed improvement (17/37, 45.9%) or stable disease course (13/37, 35.1%), whereas clinical worsening was observed in only 7 cases (7/37, 18.9%). Peripheral arthritis and uveitis showed good clinical response rates to anti-TNF (61.5% and 66.7%, respectively), whereas those of PG, axial arthropathy, aphthous stomatitis, and EN were $\leq$50% (50.0%, 50.0%, 33.3%, and 33.3%, respectively, Table 3). In a multivariate regression model adjusted for age and sex, appearance of EIM before IBD diagnosis was the only independent predictor for positive anti-TNF outcome (odds ratio 9.70, 95% confidence interval 1.04–90.04, $P = 0.046$). Details about the multivariate analysis can be found in the Supplemental Digital Content 2, Table 2 (*http://links.lww.com/MPG/A828*).

DISCUSSION

In this analysis of the PSIBDCS, we report on frequency and chronological order of appearance of EIM, the use of anti-TNF agents, and the disease course under such therapy. EIM are common in pediatric patients with IBD and can appear in nearly 30% before IBD diagnosis is established. Anti-TNF are used frequently in those patients, although they are started for the

TABLE 2. Demographics and frequency and type of extraintestinal manifestation according to inflammatory bowel disease subtype

	All, n = 55 (%)	UC, n = 12 (%)	CD, n = 39 (%)	IBD-U n = 4 (%)
Sex				
Male	34 (61.8)	6 (50.0)	26 (66.7)	2 (50.0)
Female	21 (38.2)	6 (50.0)	13 (33.3)	2 (50.0)
Age at IBD diagnosis in years	11.3 (0.8–15.7)	9.5 (2.5–15.5)	11.5 (0.8–15.4)	14.0 (12.4–15.7)
Number of EIM				
1	39 (70.9)	6 (50.0)	29 (74.4)	4 (100.0)
2	12 (21.8)	6 (50.0)	6 (15.4)	0 (0.0)
3	3 (5.5)	0 (0.0)	3 (7.7)	0 (0.0)
4	1 (1.8)	0 (0.0)	1 (2.6)	0 (0.0)
Age at first EIM in years	12.8 (3.1–17.4)	11.5 (3.8–15.4)	13.1 (3.1–17.4)	13.0 (9.3–14.9)
Time from IBD to first EIM in months	1.0 (−37.5–149.0)	5.0 (−26.0–149.0)	1.5 (−28.0–102.0)	−18.0 (−37.4–1.0)
Type of EIM				
Arthritis	26 (47.3)	6 (50.0)	19 (48.7)	1 (25.0)
Uveitis	6 (10.9)	0 (0.0)	5 (12.8)	1 (25.0)
PG	2 (3.6)	1 (8.3)	1 (2.6)	0 (0.0)
EN	5 (9.1)	1 (8.3)	4 (10.3)	0 (0.0)
Stomatitis	24 (43.6)	5 (41.7)	18 (46.2)	1 (25.0)
AS	5 (9.1)	2 (16.7)	3 (7.7)	0 (0.0)
PSC	4 (7.3)	3 (25.0)	0 (0.0)	1 (25.0)
Psoriasis	4 (7.3)	0 (0.0)	4 (10.3)	0 (0.0)
Occurrence of EIM	n = 76	n = 18	n = 54	n = 4
Before	21 (27.6)	4 (22.2)	14 (25.9)	3 (75.0)
Concomitant	12 (15.8)	3 (16.7)	9 (16.7)	0 (0.0)
After	42 (55.3)	11 (61.1)	30 (55.6)	1 (25.0)
Unknown	1 (1.3)	0 (0.0)	1 (1.9)	0 (0.0)

AS = axial arthropathy; CD = Crohn disease; EIM = extraintestinal manifestations; EN = erythema nodosum; IBD-U = inflammatory bowel disease-unclassified; PG = pyoderma gangrenosum; PSC = primary sclerosing cholangitis; UC = ulcerative colitis.

purpose of treating EIM in only a minority. Type of EIM may affect anti-TNF outcome. Best response rates were seen for peripheral arthritis and uveitis.

EIM were frequently encountered in pediatric IBD, the prevalence of 16.7% is comparable to prior data from Guariso et al (26). Prevalence is, however, considerably lower compared to the studies conducted by Dotson et al (29) and Jose et al (25), which is mainly due to our more stringent definition of EIM, because we did not include nonspecific arthralgia or other nonspecific EIM such as anemia, hepatitis, pancreatitis, or osteoporosis. In accordance to the findings of Guariso et al (26), presence of EIM at IBD onset was more frequent in the PSIBDCS compared to the adult population (8.5% vs 5%), although difference was considerably smaller (14.3% vs 7.3% in the study of Guariso). Our numbers fit well within the

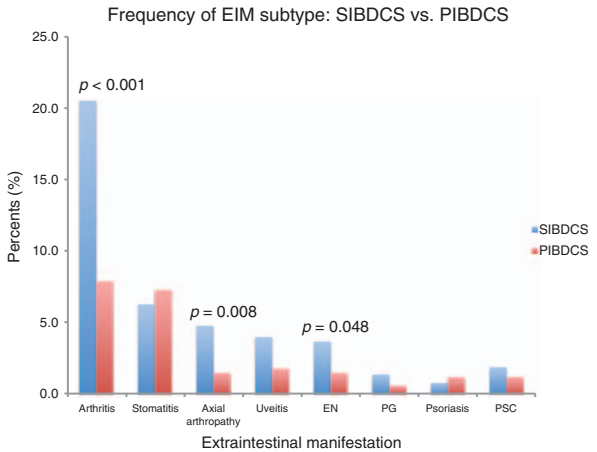

FIGURE 1. Frequency of extraintestinal manifestation (EIM) subtype among those patients presenting with EIM according to inflammatory bowel disease (IBD) cohort study (adult vs pediatric). EN = erythema nodosum; PG = pyoderma gangrenosum; PIBDCS = Pediatric Swiss IBD Cohort Study; PSC = primary sclerosing cholangitis; SIBDCS = Swiss IBD Cohort Study.

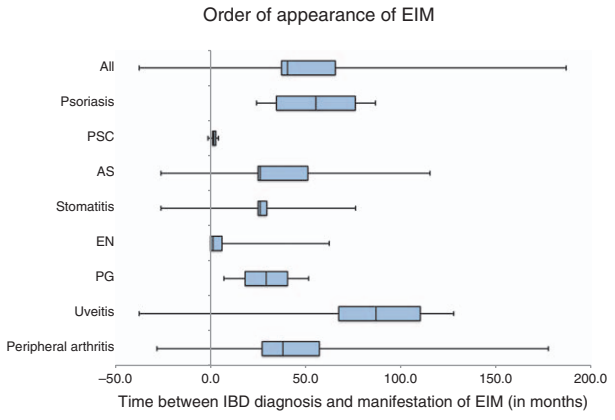

FIGURE 2. Chronological order of appearance of EIM in relation to the time of IBD diagnosis. Data are presented as horizontal boxplots. The box comprises the 25th and 75th percentile; the vertical line in the box corresponds to the median. AS = axial arthropathy; EIM = extraintestinal manifestation; EN = erythema nodosum; IBD = inflammatory bowel disease; PG = pyoderma gangrenosum; PSC = primary sclerosing cholangitis.

TABLE 3. Anti–tumor necrosis factor treatment

	All anti-TNF (n = 42)
Indication for anti-TNF	
IBD	33 (78.6)
EIM	3 (7.1)
Both	5 (11.9)
Unknown	1 (2.4)
Treated EIM	
Arthritis	16 (38.1)
Uveitis	4 (9.5)
PG	3 (7.1)
EN	4 (9.5)
Stomatitis	13 (31.0)
AS	5 (11.9)
PSC	0 (0.0)
(Unknown type)	(8)
Outcome of treated EIM	n = 37
Improvement	17 (45.9)
Stable disease	13 (35.1)
Worsening	7 (18.9)
(unknown outcome)	(16)
Anti-TNF response rates	
Arthritis	61.5% (8/13)
Uveitis	66.7% (2/3)
PG	50.0% (1/2)
EN	33.3% (1/3)
Stomatitis	33.3% (3/9)
AS	50.0% (2/4)

AS = axial arthropathy; EIM = extraintestinal manifestation; EN = erythema nodosum; IBD = inflammatory bowel disease; PG = pyoderma gangrenosum; PSC = primary sclerosing cholangitis; TNF = tumor necrosis factor.

range of 6% to 47% EIM prevalence typically reported (2,4–6,13,30). Our finding that EIM were more frequently observed in CD compared with UC patients is consistent with prior studies from adult IBD cohorts (7,8,13). Moreover, the order of frequency is consistent throughout the adult EIM literature and our data are in agreement with published observations: musculoskeletal symptoms (such as peripheral arthritis) are followed by stomatitis, ophthalmological problems (such as uveitis), and skin changes (8,25). Although the proportion of patients presenting with arthritis is higher among adult patients, order of frequency of EIM did not show a difference between the adult and pediatric patients with IBD. EIM were more likely to appear after IBD diagnosis compared to before. Nonetheless, a non-negligible proportion (up to 30%) of EIM appeared before IBD diagnosis was established. Axial arthropathy appeared before IBD diagnosis in an even higher proportion. Both findings are consistent with data from adult cohorts (7). So, clinicians should be aware of those EIM manifesting before intestinal symptoms to decrease the diagnostic delay. Our group has recently shown that such diagnostic delay is a concern (31) and that a delayed diagnosis is associated with a complicated disease course in patients with CD (32). Appearance of >1 EIM was observed only infrequently. Thus, the fact that 1 EIM seems to increase the susceptibility of developing other EIM does not seem to be true for pediatric patients (33) or latency between first and second EIM may have been too long to allow detection in children. Taken together, our data suggest that epidemiology and presentation of EIM is quite similar in the pediatric and the adult population, suggesting similar disease mechanisms.

Anti-TNF were frequently used in pediatric patients with EIM. Those agents were, however, specifically initiated for treating EIM in only a minority. So, clinical practice seems to be in accordance to current guidelines, which recommend to treat underlying IBD activity rather than EIM themselves. Nonetheless, patients with EIM were significantly more often treated with anti-TNF than those without EIM. The latter may be explained by prior findings from Vavricka et al (8) who showed that active disease is an independent risk factor for EIM in both UC and CD. Upon anti-TNF treatment, EIM showed overall response rates of nearly 50%, which depended on the underlying EIM with the best rates for peripheral arthritis and uveitis. Although direct comparisons are not possible, higher response rates to anti-TNF treatment were described in the adult population (71.8%) (34). New onset of EIM under anti-TNF treatment also has been encountered in our study. Diagnosis of psoriasis was established in 3 of the 31 anti–TNF-treated patients, which can be interpreted retrospectively as anti–TNF-induced psoriasiform skin lesion. Prevalence seems to be in accordance to that reported in the literature (35).

Our study has several strengths and some limitations as well. So far, it is one of the largest analyses of collected data evaluating frequency and occurrence of EIM in the pediatric IBD population. Furthermore, a detailed chart review revealed information about chronological appearance of EIM according to EIM subtype, the use of anti-TNF treatment and clinical response to such a therapy. The combination of physician- and patient-based questionnaire may have prevented the underreporting of EIM in our study population. We, however, used an uncontrolled, noninterventional study design, which limits interpretation of anti-TNF treatment outcome. With annual follow-up visits, important details occurring during this period of time may have been missed. This potential recall bias has, however, been limited by the combination of physician- and patient-based questionnaires. Given the fact that the Pediatric Swiss IBD cohort is not strictly population based, a selection bias may be present. For instance, patients included by private practices and community hospitals were underrepresented compared with those patients included by university hospitals (30.9% of the patients presenting with EIM were recruited in private practices or community hospitals, whereas 69.1% were recruited by physicians working in a university hospital). A clear limitation of our analysis is that—at the time of data analysis—we did not clearly differentiate between psoriasis and psoriasiform anti–TNF-associated skin lesions, which is an increasingly reported phenomenon (35). With 4 cases, prevalence of psoriasis was, however, extremely low, and 3 out of 4 cases can be retrospectively considered as anti-TNF induced as they presented under anti-TNF treatment for the first time. Because of the retrospective nature of the study, a non-negligible proportion of patients received concomitant immunomodulators and/or steroids in addition to anti-TNF, which may have led to an overestimation of anti-TNF response rates.

In summary, in a cohort of pediatric patients with IBD, EIM were frequently encountered. In up to 30% of patients, EIM appeared before IBD was diagnosed. Knowledge of these findings may result in increased awareness of underlying IBD, thereby decreasing potential diagnostic delay. Anti-TNF for the treatment of certain EIM is effective, although a substantial proportion of new EIM may present despite ongoing anti-TNF therapy. Randomized controlled trials are, however, needed in the pediatric IBD population.

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